

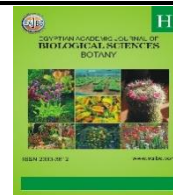
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Solvent-Dependent Phytochemical and Antioxidant Profiles of *Tamarindus indica* L (Tamarind) pulp: A Comparative Study

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ABSTRACT

Tamarindus indica L. (Tamarind) of family Fabaceae is an edible fruit native to Africa's tropical regions. This study analysed the proximate content of tamarind fruit pulp as well as compared the phytochemical and antioxidant properties using three different solvents: lime juice, honey solution, and aqueous solution. Proximate content was assayed through protocols of AOAC while phytochemical and antioxidant analyses were carried out using standard methods. The percentage of proximate content varied with ash value (4.61 ± 0.01), crude fibre (7.27 ± 0.03), crude fat/lipid (3.83 ± 0.02), crude protein (8.49 ± 0.01) while the highest content was from crude carbohydrate at 78.21 ± 0.02 %. In the qualitative phytochemical analysis, saponins, reducing sugar, terpenoids, cardiac glycosides, flavonoids steroids, and phenolic compounds were detected in all three samples while alkaloids, anthraquinones, tannins and triterpenoids were not detected. The highest concentration of reducing sugar at 255.89 ± 0.13 mg/g was detected in honey extract while phenolic compounds were detected more in the aqueous extract. In the antioxidant assay, aqueous and lime juice extracts of the tamarind fruit pulp exhibited greater scavenging potential on DPPH at dose-dependent rates. This comparative study provides insights into solvent selection for extracting bioactive compounds from tamarind pulp, emphasizing lime juice and honey as promising options. Such findings are valuable for industries involved in tamarind processing for consumption and could contribute to developing more efficient and sustainable extraction methods.

INTRODUCTION

Tamarindus indica Linn. (Tamarind), is an edible fruit native to tropical Africa and naturalized in Asia (El-Siddig, *et al.*, 2006). The genus *Tamarindus* is monotypic and belongs to the family Fabaceae. Almost all parts of the plant serve a purpose in medicine, economics, nutrition, or environmental contexts, earning it the status of a multipurpose tree. Bhadoriya *et al.* (2011) reported it as a tree that has the capacity to tolerate 5–6 months duration of dry conditions with a very minimal chance of surviving at stumpy temperatures. Tamarind is categorized into two main types, the acidic and the sweet acidic varieties. The acidic variety thrives in warm, sunny locations and is consequently more prevalent. The sweet acidic variety, however, is more delicate and sensitive to temperature changes, making it less readily accessible. Tamarind's primary value lies in its fruit, comprising predominantly of pulp and seeds.

According to report by Rao and Mathew (2012) both ripe and dry fruits of Tamarind are rich in tartaric acid, reducing sugars, pectin, tannin, fiber, and cellulose. Naeem *et al.* (2017) and Okello *et al.* (2017) also reported the pulp (fruit) and seeds as abundant sources of calcium, phosphorus and potassium along with minerals such as sodium, zinc, and iron. These phytochemicals play beneficial roles in human health, demonstrating significant antioxidant activity.

According to Toungos (2019), Tamarind for a long time has been a staple spice common to southern India. Tamarind seeds are the residual products of industries utilizing tamarind pulp as their primary material. In certain developing nations, these seeds can act as an alternative protein source, helping alleviate malnutrition concerns. It is a plant whose fruits can be exploited for sustainable development solving the concerns of SDGs 4, 7 and 8. The flowers and leaves are edible, either consumed fresh, cooked in diverse dishes, or prepared as ingredients for curries, salads, and soups (De Caluwé *et al.*, 2010 and Chimsah *et al.*, 2020). The fruit pulp is abundant in phytonutrients, particularly phenolic compounds, which possess the capability to function as effective dietary antioxidants. Narina *et al.* (2019) reported it as rich in polyphenols such as flavan-3-ols (epicatechin and 16 Tamarind 321 catechin), flavonoids (vitexin, iso-vitexin), procyanidin, triterpenes (orientin, iso-orientin) and vitamins (B3, E and C). Tamarind also possesses organic acids, such as acetic acid, citric acid, formic acid, malic acid, oxalic acid, succinic acid, and quinic acid as reported by Katsayal *et al.* (2019).

The predominant solvents for extraction from Tamarind have been ethanol and methanol (Hassim *et al.*, 2014; Highab, 2021; Ningrum *et al.*, 2024), these solvents however carry potential side effects. These include the risk of experiencing headaches, drowsiness, nausea, vomiting, and unconsciousness. Both are classified as volatile organic compounds (VOC) and pose risk of contributing to air pollution upon release into the atmosphere. Using substances such as honey, lime and water as solvents can have several merits in various applications; they are natural and safe; they do not contain harmful chemicals or toxins and they are edible (Banerjee *et al.*, 2016; Phaisan *et al.*, 2020; Birinci *et al.*, 2025). Honey and water are biodegradable and do not contribute to pollution. They are also readily available, versatile and enhance the stability of certain formulations and act as natural preservatives which help to extend the shelf life of products. It is important and necessary to use alternative solvents which are much safer for extraction. There is a lack of comparative data on the effectiveness of different solvents, including edible solvents, for the extraction of phytochemicals and antioxidants. This can be a pertinent way in addressing United Nations SDGs 2, 3 and 7 which are “No hunger; Good health and well-living; affordable and clean energy”. Therefore, the main purpose of this study is to carry out comparative studies of the phytochemical profile and antioxidant properties of *Tamarindus indica* pulp using natural solvents like lime juice, honey, and aqueous solutions as well as to determine the proximate content of the fruit pulp.

MATERIALS AND METHODS

Sample Collection:

Tamarindus indica fruits were collected from Landscape Garden in Lagos mainland in May - June, 2024 and authenticated by the curator in charge at the Lagos University Herbarium (LUH) of the University of Lagos.

Sample Extraction and Preparation:

The Tamarind fruits were transported to the laboratory in Ziploc bags. The fruits were dried in an Equitron oven for 50 mins under 30 °C. After which the seeds were separated from the fruits using sterilized blade under aseptic condition. The fruits were then pounded with a mortar and pestle to pulp. Using a weigh balance, 30 g of the pulp was

measured into a 500 ml conical flask to which 300 ml of lime solution in ratio 1:2 (lime juice to water) was added and labelled flask A. A measure of 30 g of the pulp was measured into another flask labelled B and filled with 300 ml honey solution in ratio 1:2 (honey to water). While for the aqueous extract, a third flask labelled C was filled with 30 g of the tamarind pulp and 300 ml of sterile distilled water was added. Each flask was replicated and sealed tightly with foil paper. The flasks were placed on rotary shaker under room temperature of 28 ± 2 °C for 72 h. The content of each flask was filtered using No 1 Whatman filter paper and concentrated to dryness by evaporation at ambient temperature. The crude extracts were stored at 4 °C for further analysis.

Proximate Content Assay:

Portion of the fruit pulp was used for proximate content analysis. The total ash content, crude protein, moisture content, crude lipids and crude fibre were analyzed using Association of Official Analytical Chemists (AOAC, 2012) protocol while the carbohydrate content was estimated by subtracting the sum percentage of moisture, ash, protein, lipids and fibre from 100 % following Thiex (2009) and Maisarah *et al.* (2014) methods.

Preliminary Phytochemical Analysis:

Both qualitative and quantitative phytochemical analysis were carried out according to standard protocols of Dhani, (2012), Hossain *et al.* (2013) and Auwal *et al.* (2014).

Determination of Antioxidant Activities:

Free Radical Scavenging using DPPH Assay:

A measure of 1 ml of 0.1 mM of DPPH in ethanol solution was pipetted into 1 ml of extract in water at different concentrations (10-100 µg/ml). The mixture was then gently agitated and allowed to stand for 30 min at 25 °C. An UV-Visible Spectrophotometer was then used to measure the absorbance at 517 nm. The percent DPPH scavenging effect was calculated using the equation:

$$\text{DPPH Scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A₀ is the absorbance of the control and A₁ is the absorbance in the presence of the standard extract. The IC₅₀ value represented the concentration of the compounds that caused 50 % inhibition of DPPH radical formation. This is as described by Kedare and Singh, (2011).

Statistical Analysis:

The data were presented as mean and standard deviation while Statistical Package for Social Science (SPSS) version 22 was used for the data analysis. All experiments were carried out in triplicate.

RESULTS

Qualitative Phytochemical Components of Tamarind Fruit Pulp:

The effectiveness of extraction solvents can be measured by their ability to extract bioactive compounds from sampled materials. The qualitative analysis of this study showed the detection of saponins, reducing sugar, terpenoids, cardiac glucosides, steroids, phenolic compounds, and flavonoids in the test samples using the three solvents, while alkaloids, anthraquinones, triterpenoids and tannins were not detected in the sampled material. Saponins were detected more in the lime juice extract, while reducing sugar and phenolic compounds were detected more in lime juice and honey solution extracts. This is presented in Table 1.

Table 1: Qualitative phytochemical components of Lime juice, Honey solution and Aqueous Extracts of Tamarind Fruit Pulp.

Test	Method	Lime juice	Honey solution	Aqueous
Alkaloids	Mayer's Test	-	-	-
	Wagner's Test	-	-	-
Flavonoids	Shinoda's Test	++	++	+
Saponins	Frothing Test	+++	+	+
Cardiac glycosides	Keller Killani's Test	+	+	+
Anthraquinones	Borntrager's Test	-	-	-
Triterpenoids	Lieberman-Buchard's	-	-	-
Terpenoids	Lieberman-Buchard's	+	+	+
Phenolic Compounds	Lead acetate Test	+++	+++	+
Tannins	Ferric chloride Test	-	-	-
Reducing Sugar	Fehling's Test	+++	+++	++
Steroids	Salkowski's Test	+	+	+

Key: Heavily detected: +++; detected: ++; slightly detected: +; not detected: -

Quantitative Phytochemical Components of Tamarind Fruit Pulp:

A quantitative analysis further elucidates the efficiency of extraction solvents by providing numerical data on the concentration of specific phytochemical compounds in the tamarind extracts. From the result presented in Table 2, reducing sugar showed the highest concentration in all solvents with honey solution extract having the highest value of 255.89 ± 0.13 mg/g while steroids showed the lowest concentration across the extracts with honey extract having the lowest of 0.24 ± 0.01 mg/g.

Table 2: Quantitative phytochemical components of lime juice, honey solution and aqueous extracts of tamarind fruit pulp.

Phytoconstituents	mg/g of dry extract		
	Lime juice	Honey solution	Aqueous
Reducing Sugar	101.75 ± 0.24	255.89 ± 0.13	72.22 ± 0.16
Phenolic compound	3.00 ± 0.01	2.87 ± 0.01	4.76 ± 0.02
Flavonoids	9.33 ± 0.43	5.77 ± 0.02	4.76 ± 0.01
Steroids	0.52 ± 0.00	0.24 ± 0.01	0.74 ± 0.01

Values are express as Mean $\pm$ SD

Proximate Contents of *Tamarindus indica* Fruit Pulp:

The proximate content analysis was done in triplicate. The result showed that the values of moisture, ash content, crude fibre, crude fat/lipid, crude protein were low compared to the Carbohydrate content which was highest at 78.21 ± 0.02 %. This result is as represented in Table 3.

Table 3: Proximate content of Tamarind Fruit pulp.

Proximate	Moisture content	Ash content	Crude fibre	Crude fat/lipid	Crude protein	Crude carbohydrate	Energy-calories (kcal)
Results (%)	8.66 ± 0.02	4.16 ± 0.01	6.35 ± 0.02	3.37 ± 0.01	6.14 ± 0.01	71.32 ± 0.04	343.80 ± 0.25

Values are express as Mean $\pm$ SD

Antioxidant Activities:**DPPH Radical Scavenging Activity of *Tamarindus indica* Fruit Pulp in Lime juice, Honey solution and Aqueous Extracts:**

The current study evaluated the potency of tamarind fruit pulp obtained using different solvents to scavenge DPPH radicals. The results indicated that aqueous extract exhibited the highest DPPH radical scavenging activity at lower concentrations of between 10-50 $\mu\text{g/ml}$. Lime juice extract exhibited moderate, dose-dependent antioxidant activity. This began from 3.41 % at 10.00 $\mu\text{g/ml}$ and consistently increased in inhibition to 23.63 % at 250.00 $\mu\text{g/ml}$. While lime juice at higher concentrations of 100.00 and 250.00 $\mu\text{g/ml}$ showed greater scavenging potential as potent antioxidants. However, all extracts were below the IC_{50} average threshold compared to the ascorbic acid, which served as the control. The result is as presented in Figure 1.

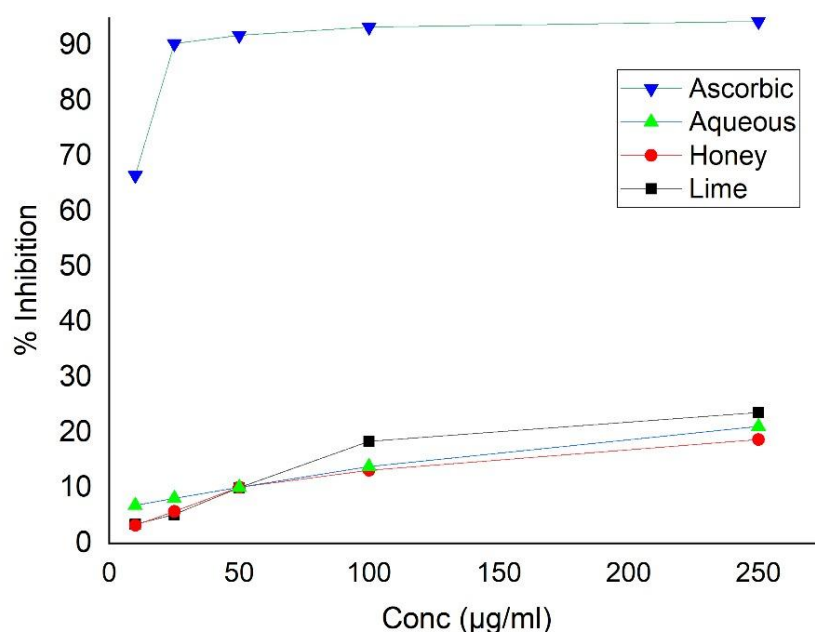


Fig. 1: DPPH Radical Scavenging activity (% Inhibition) Values are express as Mean $\pm$ SD

DISCUSSION

This study was carried out to evaluate the proximate contents as well as compare the antioxidant potential and phytochemical constituents of Tamarind fruit pulp extracted using natural solvents of lime juice, honey and aqueous solutions. The qualitative analysis for phytochemicals showed varied amounts of saponins, reducing sugars, phenolic compounds, flavonoids, terpenoids, steroids, and cardiac glycosides across the solvents. The effectiveness of these substances (lime juice and honey) as solvents can be attributed to the polarity and chemical properties that enable the dissolution of various bioactive compounds. Although, they are not as efficient as organic solvents like ethanol and methanol which can extract both polar and non-polar compounds from plants (Lee *et al.*, 2024). Shaik *et al.* (2022) review on lemon juice as a biocatalyst and green solvent has shown that members of the citrus family are being exploited as a solvent, while Banerjee *et al.* (2016) also demonstrated lemon juice as a base solvent for the extraction of pectin from mango peels. Honey demonstrated moderate efficiency in extracting certain phytochemicals. It was particularly effective in extracting reducing sugars and phenolic compounds. This correlates Phaisan *et al.* (2020) on the use of honey in extracting bioactive compounds from *Pueraria candollei* var. *mirifica*. They also noted that honey contains β -glucosidase, as such can be

used as a solvent for the extraction of bioactive compounds from herbs serving as an eco-friendly alternative to traditional solvents. Honey also showed moderate efficiency in extracting saponins, flavonoids, terpenoids, and steroids, making it a viable alternative extraction solvent depending on the target compounds. The aqueous extract showed efficiency in extracting detectable amounts of certain bioactive compounds such as reducing sugars, phenolic compounds, flavonoids, and steroids, from the sampled Tamarind fruit pulp, indicating some utility in specific extractions. Water as a strong solvent is classified in the same category as ethanol and methanol as noted by Lee *et al.* (2024). Hassim *et al.* (2014) noted methanol as the most commonly used solvent of extraction with high yield but its toxic characteristics comes into contention when it is used in food and pharmaceutical applications. However, water is known as a safe, versatile universal solvent especially for hydrophilic compounds.

In the proximate content analysis, it was observed that Tamarind fruit pulp had low ash, crude fiber, protein, and fat/lipid contents. The moisture content was slightly high while the largest chunk was the carbohydrate content. Several works have also shown the quantities of the proximate content in Tamarind fruit pulp to be in the observed range as obtained in our study. Abubakar *et al.* (2024) report on the fruit pulp of tamarind observed average compositions of 15.05-15.30 % (moisture), 4.10-5.05 % (ash), 13.70-14.95 % (lipid), 8.72-8.75 % (protein), 5.50-6.00 % (fiber) and 56.07-58.15 % (carbohydrate) similar to our observations. Earlier observations by Ishaku *et al.* (2016) and Rana and Sharma (2018) also showed similar results for the proximate content. They also noted that the fruit pulp is a good source of essential nutrients like carbohydrates and proteins.

The DPPH radical scavenging activity assay is widely used to evaluate the antioxidant capacity of plant-based or natural substances. Aqueous extract showed the highest DPPH radical scavenging activity at low concentrations of between 10-50 µg/ml, indicating potent antioxidant properties. Lime juice extract had a steady increase along the concentration gradient. It showed more antioxidant power than honey and aqueous solution. This steady increase could be attributed to the presence of active antioxidant compounds such as vitamin C, flavonoids, or phenolic compounds found in citrus, as noted by Phucharoenrak *et al.* (2022). It was noticed that the honey extract had low antioxidant potential. This lower radical scavenging capacity is probably due to the lower concentration of phenolic compounds or possible sugar interference in the test. Phenolic compounds are fundamental ingredients in honey where their concentration greatly impacts the scavenging potential of honey. Based on their studies on different varieties of honey, Alzahrani *et al.* (2012) noted that the higher the concentration of the phenolic compounds in honey the higher its scavenging potential. While Gulcin and Alwasel (2023) study on DPPH radical scavenging assay showed that sugar can interfere with reaction mechanism by either reacting with radicals or altering the reaction environment. This may be the case in the present study as honey extract of the Tamarind fruit pulp showed high sugar concentration. None of the extracts reached the IC₅₀ even at high concentrations. This probably could be due to low quantity of the Tamarind fruit pulp. The natural samples showed dose-dependent activity much weaker than the ascorbic acid standard. Ugwuona and Onweluzo (2013) reported that water and ethanol extracts of Tamarind fruit pulp scavenged DPPH in a dose-dependent manner. They also stated a decrease in value as the concentration of Tamarind pulp increases. Suggesting further studies for possible utilization as a natural antioxidant in drug, food and cosmetic production.

The high antioxidant activity of lime juice suggests its potential effectiveness in neutralizing free radicals. This potential can be the synergetic effects of the bioactive components as observed in the phytochemical analysis.

Using substances like honey, lime juice and aqueous solution as solvents can have several merits in various applications. They are natural and safe, lack harmful chemicals or

toxins, eco-friendly, readily available, versatile and edible (Banerjee *et al.*, 2016; Phaisan *et al.*, 2020; Phucharoenrak *et al.*, 2022). They are biodegradable, do not contribute to pollution and acts as natural preservatives which help extend the shelf life of products. There is a lack of comparative data on the effectiveness of different solvents, including edible solvents, for the extraction of phytochemicals and antioxidants.

The choice of extraction solvent has a significant impact on the phytochemical composition, antioxidant activity, and nutritional value of tamarind extracts. Lime juice has the highest antioxidant potential among the natural samples, while none approached a 50 % inhibition. They can contribute to antioxidant intake by increasing their concentration and carrying out further purification processes to ascertain the specific bioactive components.

Declarations:

Ethical Approval: There is no need for ethical issues because no human, animal or plant subject was used in this study.

Conflict of Interests: The authors declare that there are no conflicts of interest related to this article.

Authors' Contributions: I hereby verify that all authors mentioned on this title page have contributed to the planning and execution of this article. All authors have carefully read the manuscript, attested to the veracity and correctness of the data and its interpretation, and have given their approval for submission.

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Availability of Data and Materials: Upon reasonable request, the corresponding author will make all datasets analyzed and described in this paper available.

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